Docket No.: 21058/1206447-US2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Xing Su et al.

Patent No.: 7,238,477

Issued: July 3, 2007

For: METHODS TO INCREASE NUCLEOTIDE SIGNALS BY RAMAN SCATTERING

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 CFR 1.323

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

On March 20, 2008, the undersigned mistakenly filed a 312 Amendment in order to correct an error in the claims. This correction was suggested by the Examiner during a telephonic interview on March 19, 2008 with the undersigned. Patentee now respectfully requests that the correction to the claims be granted via a Certificate of Correction.

Claim 7 recites "sequentially releasing nucleotides" in step (d) and "identifying the released unlabeled nucleotides" in step (e). Arguably, the there is no antecedent basis for "the released *unlabeled* nucleotides." During the interview of March 19, 2008, the Examiner suggested to the undersigned to correct claim 7 by deleting the word ***unlabeled***. Accordingly, Patentee requests that claim 7 be corrected to remove the word ***unlabeled***.

The error now sought to be corrected is inadvertent, the correction of which does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting the correction. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Payment in the amount of $100.00 is attached for the filing of this Request. The Commissioner is authorized to charge any deficiency of up to $300.00 or credit any excess in this fee to Deposit Account No. 04-0100.

Dated: April 11, 2008

Respectfully submitted,

By / Raj S. Davé /
Raj S. Davé
Registration No.: 42,465
DARBY & DARBY P.C.
P.O. Box 770
Church Street Station
New York, New York 10008-0770
(202) 639-7515
(212) 527-7701 (Fax)
Attorneys/Agents For Intel Corporation

PTO/SB/44 (09-07)
Approved for use through 08/31/2010. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	7,238,477
APPLICATION NO.	:	10/660,902
ISSUE DATE	:	July 3, 2007
INVENTOR(S)	:	Xing Su et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 1, Line 15, after the word "released", delete "unlabeled"

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Raj S. Dave, Esq.
DARBY & DARBY P.C.
P.O. Box 770
Church Street Station
New York, New York 10008-0770

The primer-template is incubated with modified T7 DNA polymerase (United States Biochemical Corp., Cleveland, Ohio). The reaction mixture contains unlabeled deoxyadenosine-5'-triphosphate (dATP) and deoxyguanosine-5'-triphosphate (dGTP), digoxigenin-labeled deoxyuridine-5'-triphosphate (digoxigenin-dUTP) and rhodamine-labeled deoxycytidine-5'-triphosphate (rhodamine-dCTP). The polymerization reaction is allowed to proceed for 2 hours at 37° C. After synthesis of the digoxigenin and rhodamine labeled nucleic acid **13**, the template strand is separated from the labeled nucleic acid **13**, and the template strand, DNA polymerase and unincorporated nucleotides are washed out of the reaction chamber **11**.

Exonuclease **15** activity is initiated by addition of exonuclease III **15** to the reaction chamber **11**. The reaction mixture is maintained at pH 8.0 and 37° C. As nucleotides **16** are released from the 3' end **17** of the nucleic acid **13**, they are transported by microfluidic flow down the flow path **12** past the detection unit **18**.

Example 6

Nucleic Acid Sequencing Using Covalent Attachment to Nanoparticles

Another exemplary embodiment of the invention is disclosed in FIG. **2**. Nucleotides **130** are released from a nucleic acid by exonuclease activity as discussed above. In certain embodiments of the invention, the nucleotides **130** are unlabeled. Unlabeled nucleic acids directly purified from any organ, tissue and/or cell sample or obtained by known cloning methods may be sequenced using exonuclease treatment. Released nucleotides **130** travel down a microfluidic channel **110**.

The released nucleotides **130** are mixed with silver nanoparticles **140**, prepared according to Lee and Meisel (*J. Phys. Chem.* 86:3391–3395, 1982). The nanoparticles are 5 to 200 nm in size. Prior to exposure to nucleotides **130**, surface-modified nanoparticles **140** are coated with a silane, such as 3-glycidoxypropyltrimethoxysilane (GOP), a reactive linker compound. GOP contains a terminal highly reactive epoxide group. The silanized nanoparticles **140** are mixed with nucleotides **130** and allowed to form covalent cross-links with the nucleotides **130**. The nucleotide-nanoparticle complexes **150** pass through a flow through cell **170** and are identified by SERS, SERRS and/or CARS using a Raman detection unit **180**. Because of the close proximity of the nucleotides **130** to the nanoparticles **140**, the Raman signals are greatly enhanced, allowing detection of single nucleotides **130** passing through the flow-through cell **170**.

Example 7

Apparatus for Nucleic Acid Sequencing

FIG. **3** shows another exemplary embodiment of the invention. A DNA sequencing apparatus **210** comprises a reaction chamber **220** in fluid communication with an influx channel **230** and an efflux channel **240**. Fluid movement may be controlled through the use of one or more valves **250**. A microfluidic channel **260** is also in fluid communication with the reaction chamber **220**. Nucleotides released from one or more nucleic acids by exonuclease activity exit the reaction chamber **220** through the microfluidic channel **260**. The nucleotides are mixed with nanoparticles that move through a nanoparticle channel **270** in fluid communication with the microfluidic channel **260**. Covalent attachment of nucleotides to nanoparticles occurs within an attachment channel **280**. The covalently bound nucleotide-nanoparticle complexes pass through a flow-through cell **290** where the nucleotides are identified by a Raman detection unit **300**. The detection unit **300** comprises a laser **320** and Raman detector **310**. The laser emits an excitation beam **330** that excites nucleotides within the flow-through cell **290**. Excited nucleotides emit a Raman signal that is detected by the Raman detector **310**.

In certain embodiments of the invention, nanoparticles may be recovered in a recycling chamber **340**. The nanoparticles are chemically treated, for example with acid solutions, and then washed to remove bound nucleotides, linker compounds and any other attached or adsorbed molecules. The nanoparticles may be recycled to a nanoparticle reservoir **370** via a recycling channel **360**. In some embodiments of the invention, nanoparticles may be coated with a linker compound, such as GOP, in the recycling channel **360** and/or the nanoparticle reservoir **370**. Waste effluent is removed from the recycling chamber **340** via a waste channel **350**.

All of the METHODS and APPARATUS disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. It will be apparent to those of skill in the art that variations may be applied to the METHODS and APPARATUS described herein without departing from the concept, spirit and scope of the claimed subject matter. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the claimed subject matter.

What is claimed is:

1. A method comprising:

a) obtaining nucleotides covalently linked to gold or silver, or gold or silver nanoparticle(s), wherein the nucleotide and nanoparticles are linked via a terminal reactive cross-linking group, selected from the group consisting of epoxide groups, azido groups, triazine groups, arylazido groups and diazo groups;

b) synthesizing one or more nucleic acid molecules comprising the gold or silver, or gold or silver nanoparticles;

c) immobilizing the nucleic acid molecule of step (b) on a solid substrate;

d) sequentially releasing nucleotides from one end of one or more nucleic acid molecules via an exonuclease;

e) identifying the released unlabeled nucleotides in a buffer comprising an alkali-metal halide salt by Raman spectroscopy; and

f) determining the sequence of the nucleic acid molecule.

2. The method of claim **1**, wherein single molecules of nucleotides are identified by Raman spectroscopy.

3. The method of claim **2**, wherein a single nucleic acid molecule is sequenced.

4. The method of claim **1**, wherein multiple nucleic acid molecules of the same sequence or multiple nucleic acid molecules of different sequences are sequenced simultaneously.

5. The method of claim **1**, wherein the alkali-metal halide salt is selected from the group consisting of MgCl, CaCl. NaF, KBr, LiI, and LiCl.